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Description of complete DNA sequence of two plasmids from the nukacin ISK-1 producer, *Staphylococcus warneri* ISK-1

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Abstract

We report the whole DNA sequence of two plasmids, pPI-1 (30.2 kb) and pPI-2 (2.8 kb). These plasmids are from *Staphylococcus warneri* ISK-1, which produces a lantibiotic, nukacin ISK-1. Curing of pPI-1 resulted in a loss of bactericidal activity in the culture supernatant and the host's immunity to nukacin ISK-1, suggesting that the biosynthetic genes of the bacteriocin are encoded by pPI-1. Based on the results of a homology search of each open reading flame, pPI-1 is comprised of the following four distinct regions: (1) the nukacin ISK-1 biosynthesis and immunity gene cluster, (2) the thioredoxin gene cluster, (3) the replication region, and (4) a region of *Staphylococcus epidermidis* ATCC 12228, highly homologous to pSE-12228-05. Gene organization in the nukacin ISK-1 biosynthesis and immunity gene cluster is different from that in other lacticin-481 type gene clusters. The features of the replication protein encoded in the replicating region are somewhat different from other staphylococcus theta-replicating plasmids. pPI-2 comprised a disinfectant resistant gene, *qacC*, and the whole DNA sequence showed significant similarity to those of other *qacC* plasmids such as pSK108, suggesting that pPI-2 belongs to the *qacC* plasmid group.

Keywords: *Staphylococcus warneri*; Plasmid; Lantibiotic; Bacteriocin; Nukacin ISK-1; *qacC*

1. Introduction

Bacteriocins are ribosomally synthesized peptides produced by bacteria and generally show

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antibacterial spectra against closely related bacterial species or different strains in the same species (Jack et al., 1995; Klaenhammer, 1993; Nes et al., 1996). Because of their useful characteristics, notably for industrial purposes, bacteriocins are in great demand. Genes involved in the production of bacteriocins are often encoded in plasmids (Klaenhammer, 1993). Recently, the 60-kb conjugative plasmid pMRC01 from *Lactococcus lactis* subsp. *lactis* DPC3147, comprising the biosynthetic genes for lacticin 3147, was completely sequenced, and the functions of the plasmid were discussed (Dougherty et al., 1998). The sequence analysis of pMRC01 revealed that some functional regions for conjugal transfer and phage resistance were present, in addition to the bacteriocin gene cluster in the plasmid. Also, insertion sequence elements for integration events were found in the plasmid. These results, obtained from DNA sequence analysis of bacteriocin plasmids, could give us insight into the evolution of the functional plasmid, and how to make good use of the plasmid for industrial purposes.

A novel bacteriocin, nukacin ISK-1, is produced by *Staphylococcus warneri* ISK-1 (Kimura et al., 1997, 1998). Nukacin ISK-1 can be classified into lacticin-481 type lantibiotics, containing unusual amino acids such as lanthionine and dehydrated amino acids (Jung, 1991; McAuliffe et al., 2001; Sahl et al., 1995). DNA sequence research and homology search analysis revealed that the gene cluster of this bacteriocin consists of at least seven genes, including *nukA*, *-M*, *-T*, *-F*, *-E*, and *-G* and two additional open reading fames (ORFs),[1] ORF1 and ORF7 (Aso et al., 2004; Sashihara et al., 2000). The *nukA*, *-M*, and *-T* genes encode the pre-nukacin ISK-1 structure gene, a putative modification enzyme for unusual amino acids, and a putative ABC transporter for the secretion of matured nukacin ISK-1, respectively. Products translated from *nukF*, *-E*, and *-G* genes contribute to the immunity to nukacin ISK-1. The function of ORF1 is not yet clear, although its amino acid sequences showed similarity to cognate response regulators. The ORF7 product showed sequence similarity to the ORF4 product encoded in the gene cluster of a lacticin-481 type lantibiotic, butyrivibriocin OR79A, from *Butyrivibrio fibrisolvens* OR79 (Kalmokoff et al., 1999) and a putative immunity-related protein, RumH encoded on the locus of a lacticin-481 type lantibiotic, ruminococcin A, from *Ruminococcus gnavus* E1 (Gomez et al., 2002).

Staphylococcus. warneri ISK-1 has two plasmids, pPI-1 and pPI-2, approximately 30.2 and 2.8 kb in size, respectively. In this study, we describe the complete DNA sequence of both plasmids. pPI-1 consists of four distinct regions: the thioredoxin gene cluster, a replication region showing features of theta-replicating plasmid, a region highly homologous to a cryptic plasmid found in *Staphylococcus epidermidis* ATCC 12228, and the nukacin ISK-1 gene cluster. pPI-2 showed typical features of a disinfectant resistant plasmid known as *qacC* plasmid. Together with the sequence information, we discuss the functions and evolutionary aspects of these two plasmids.

2. Materials and methods

2.1. Bacterial strains, media, and plasmids

Staphylococcus warneri ISK-1 producing nukacin ISK-1 was grown in MRS medium (Oxoid, Hampshire, United Kingdom) at 37 °C. *Escherichia coli* JM109 (Yanisch-Perron et al., 1985) was grown in LB medium (Sambrook et al., 1989) at 37 °C. When needed, 40 mg/L of ampicillin was added to the medium. The plasmid, pUC18 (Toyobo, Osaka, Japan), was used to clone fragments of pPI-1 and pPI-2.

2.2. Plasmid-curing experiment

For curing pPI-1, *S. warneri* ISK-1 was grown at 37 °C for 12 h in MRS medium supplemented with acriflavine, at a concentration of 25 mg/L when A_{562} reached 0.5. For curing pPI-2, *S. warneri* ISK-1 was grown at 40 °C in MRS medium for 24 h. After the cultivations, the cultures were diluted and spread on MRS agar, then incubated at 37 and 30 °C, respectively, for pPI-1 and

[1] *Abbreviations used:* ORF, open reading frame; TrxA, thioredoxin; TrxB, thioredoxin reductase; RC plasmid, rolling-circle plasmid.